# Applicability of AFM in cancer detection

**To the Editor** — Using atomic force microscopy (AFM), Cross *et al.*[1] showed that cancer cells from patients are about 70% less stiff than normal cells. Seven different clinical samples, each consisting of eight normal and eight malignant cells, were measured and from the reported results, one can deduce that three or four force curves were recorded at the same position for each single cell. Cross *et al.* conclude that nanomechanical analysis may be a potentially useful technique for detecting cancers in the clinic.

The correlation between deformability of cells and diseases is well-known[2] and has been evaluated in cell filtration[3] and cell 'poking'[4] experiments. Developments in AFM[5] have allowed cancer detection at the single-cell level by mechanical means; coupled with routine morphological analysis of cells on histological specimens, large differences between cancerous and normal cells can be identified with more certainty. However, for cell stiffness to be used as an indicator of cancer the following considerations should be put forward.

Measurements on a significant number of cells are required for a statistically meaningful set of data. This is particularly important because only a single sample is typically drawn from patients, and as such the possibility of repeating the analysis and the probability of obtaining identical cancerous cells is low. Moreover, factors such as patient diet, medication and smoking habits can also affect the cell stiffness[6]. To determine the appropriate number of cells to measure, one needs to take into account the stiffness variations in a single cell and also in the population of cancerous cells in the patient sample. The way to do this is to consider cells as heterogeneous objects and to take multiple indentations at different locations on each cell and then calculate Young's modulus — the quantitative measure of the elastic properties of cells — for each cell separately.

Further to this, it would be necessary to study whether consecutive probing of the cell surface during the measurements can influence their elastic properties over time. The static measurements by AFM may not be as sensitive as interference methods[7] in detecting rapid, dynamic changes within the cytoskeleton of the cell. However, if the cytoskeleton remodelling has a significant influence, it should propagate to the next probed location. Therefore, one way to check the influence of prolonged poking on the cell is to monitor the change in Young's modulus over time during the measurements.

Several sources of errors that could potentially limit the use of AFM for cancer detection must be pointed out. First, although the Hertz model[8] is widely used to estimate Young's modulus with relatively good accuracy, the model assumes the living cell is an infinitely thick, purely elastic, isotropic and homogeneous material even though a cell is a highly heterogeneous material. This assumption makes it extremely difficult to obtain absolute values of Young's modulus. Moreover, the model approximates the real shape of the AFM tip by a cone or a paraboloid. One should remember that indentation depth is determined indirectly from AFM measurements, and therefore, small indentations are burdened with large errors arising from the uncertainty of the contact point, from the noise fluctuations of the baseline or from the change in the contact area. All these factors may influence the values of Young's modulus and given the heterogeneous characteristic of the cell, it is difficult to assume that taking a few measurements at one position (the centre of the cell) will satisfactorily reflect the mechanical properties of the entire cell.

In conclusion, the elasticity measurements of single cells should be carefully processed taking into account the limitations of AFM, and rigorous statistical tests should be used before this method can be reliably used for cancer diagnosis. ❐


References

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**Cross *et al.* reply** — Taking measurements on more cells is no doubt a necessity. However, mapping the cell by taking multiple indentations at different locations on the cell introduces variations, because as one goes off-centre and closer to the cell edge, the contact area of the tip changes and the Hertz model becomes less accurate (Fig. 1). Further away from the cell centre and closer to the edge of the cell where the focal adhesions — anchorage points of cells — are found, the substrate can exert effects on the cell that make it appear stiffer. This is well-understood and is the reason most studies probe cell-stiffness over the central region of the cell and within a certain indentation limit (for example, ~10% of the cell's height)[1]. In the region where Hooke's law is obeyed, we used the Hertz model, which is sufficient to evaluate the relative stiffness of the cell. For very thin samples, we regularly use the thin-film Hertz model, which has been applied to thin polymers, Matrigels and other materials on a stiff substrate.

We showed recently that the prolonged 'poking' of single cells (200 s) can result in the remodelling of the cell cytoskeleton[2] and thus, for all our studies, we limit the probing to a maximum of about five times to avoid these effects.

Finally, in our recent follow-up publication[3], we showed that measuring the centre of cytospun cells obtained from the body fluid of cancer patients, which are approximately spherical, gave similar results to flattened cells as reported in our original publication in *Nature Nanotechnology*[4]. Although patient samples may not be entirely clonal — that is, having identical characteristics — such